

**AMENDMENTS TO THE CLAIMS**

1. (Currently Amended) A method for preparing a cytotoxic lymphocyte characterized in that the method which comprises: the step of carrying out at least one of induction, maintenance and expansion of

expanding a cytotoxic lymphocyte in the presence of fibronectin, a fibronectin fragment thereof or a mixture thereof,

wherein the fibronectin fragment is

i) a polypeptide comprising at least one of the amino acid sequences of SEQ ID NOS: 1 to 19, or

ii) a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:

a) glycine, alanine;

b) valine, isoleucine, leucine;

c) aspartic acid, glutamic acid, asparagine, glutamine;

d) serine, threonine;

e) lysine, arginine; and

f) phenylalanine, tyrosine.

2. (Currently Amended) The method according to claim 1, wherein the prepared

cytotoxic lymphocyte highly expresses an interleukin-2 receptor as compared to at a higher level than a cytotoxic lymphocyte obtained by the method for preparing a cytotoxic lymphocyte prepared in the absence of a fibronectin, a fragment thereof or a mixture thereof.

3. (Currently Amended) The method according to claim 1, wherein the prepared cytotoxic lymphocyte ~~contains CD8 positive cell in a higher ratio as compared to expresses more CD8 than~~ a cytotoxic lymphocyte obtained by the method for preparing a cytotoxic lymphocyte prepared in the absence of a fibronectin, a fragment thereof or a mixture thereof.

4. (Currently Amended) The method according to any one of claims 1 to 3, wherein the prepared cytotoxic lymphocyte highly maintains cytotoxic activity ~~as compared to longer than~~ a cytotoxic lymphocyte obtained by the method for preparing a cytotoxic lymphocyte prepared in the absence of a fibronectin, a fragment thereof or a mixture thereof.

5. (Currently Amended) The method according to claim 1, wherein said fibronectin, a fragment thereof or a mixture thereof is immobilized [[in]] on a solid phase.

6. (Currently Amended) The method according to claim 5, wherein the solid phase is a cell culture equipment vessel or a cell culture carrier.

7. (Currently Amended) The method according to claim 6, wherein the cell culture equipment vessel is a petri dish, a flask or a bag, and the cell culture carrier is beads, a membrane

or a slide glass.

8. (Withdrawn - Currently Amended) The method according to ~~any one of~~ claim 1, wherein ~~at least one of induction, maintenance and expansion of~~ expanding a cytotoxic lymphocyte is ~~carried out~~ performed in a cell culture medium ~~containing~~ comprising said fibronectin, a fragment thereof or a mixture thereof.

9. (Cancelled)

10. (Currently Amended) The method according to claim [[9]] 1, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

11. (Cancelled)

12. (Currently Amended) The method according to claim 1, comprising ~~carrying out at least one of induction, maintenance and expansion of~~

expanding a cytotoxic lymphocyte in a cell culture in the presence of said fibronectin, a fragment thereof or a mixture thereof in a cell culture equipment containing a medium,

wherein ~~the method satisfies any one of the conditions of~~ at least (a) or (b) is true:

- (a) a ratio of the number of cells present at the initiation of the cell culture to a cell culture area in the cell culture equipment being is 1 cell/cm<sup>2</sup> to  $5 \times 10^5$  cells/cm<sup>2</sup>; and
- (b) a concentration of cells in a medium at the initiation of the cell culture being is from

1 cell/ml to  $5 \times 10^5$  cells/ml.

13. (Cancelled)

14. (Withdrawn) A cytotoxic lymphocyte obtained by the method of claim 1.

15. (Withdrawn) A medicament comprising as an effective ingredient a cytotoxic lymphocyte obtained by the method of claim 1.

16. (Withdrawn) An agent for enhancing an interleukin-2 receptor expression of a cell, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereto.

17. (Withdrawn) The agent according to claim 16, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

18. (Withdrawn) The agent according to claim 17, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

19. (Withdrawn) The agent according to claim 17, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

20. (Withdrawn) An agent for improving a ratio of CD8-positive cell in a lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

21. (Withdrawn) The agent according to claim 20, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

22. (Withdrawn) The agent according to claim 21, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

23. (Withdrawn) The agent according to claim 21, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

24. (Withdrawn) An agent for improving or maintaining cytotoxic activity in a cytotoxic lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

25. (Withdrawn) The agent according to claim 24, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

26. (Withdrawn) The agent according to claim 25, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

27. (Withdrawn) The agent according to claim 25, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

28. (Currently Amended) A method for increasing expression of an interleukin-2 receptor in a cytotoxic lymphocyte, ~~characterized in that the method which comprises: the step of carrying out at least one of induction, maintenance and expansion of~~ expanding a cytotoxic lymphocyte in the presence of ~~fibronectin~~, a fibronectin fragment thereof or a mixture thereof, thereby increasing expressing of interleukin-2 in a cytotoxic

lymphocyte,

wherein the fibronectin fragment is

i) a polypeptide comprising at least one of the amino acid sequences of SEQ ID NOS: 1 to 19, or

ii) a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:

a) glycine, alanine;

b) valine, isoleucine, leucine;

c) aspartic acid, glutamic acid, asparagine, glutamine;

d) serine, threonine;

e) lysine, arginine; and

f) phenylalanine, tyrosine.

29. (Currently Amended) A method for improving a ratio increasing the number of CD8-positive [[cell]] cells in a cytotoxic lymphocyte population, characterized in that the method which comprises: the step of carrying out at least one of induction, maintenance and expansion of

expanding a cytotoxic lymphocyte in the presence of fibronectin, a fibronectin fragment thereof or a mixture thereof, thereby increasing the number of CD-8 positive cells in a cytotoxic lymphocyte population,

wherein the fibronectin fragment is

- i) a polypeptide comprising at least one of the amino acid sequences of SEQ ID NOS: 1 to 19, or
  - ii) a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:
    - a) glycine, alanine;
    - b) valine, isoleucine, leucine;
    - c) aspartic acid, glutamic acid, asparagine, glutamine;
    - d) serine, threonine;
    - e) lysine, arginine; and
    - f) phenylalanine, tyrosine.

30. (Currently Amended) A method for improving or maintaining cytotoxic activity in a cytotoxic lymphocyte, ~~characterized in that the method which comprises: the step of carrying out at least one of induction, maintenance and expansion of~~  
~~expanding a cytotoxic lymphocyte in the presence of fibronectin, a fibronectin fragment thereof or a mixture thereof, thereby improving or maintaining cytotoxic activity in a cytotoxic lymphocyte.~~

wherein the fibronectin fragment is

- i) a polypeptide comprising at least one of the amino acid sequences of SEQ ID NOS: 1

to 19, or

ii) a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:

a) glycine, alanine;

b) valine, isoleucine, leucine;

c) aspartic acid, glutamic acid, asparagine, glutamine;

d) serine, threonine;

e) lysine, arginine; and

f) phenylalanine, tyrosine.

31. (Currently Amended) The method according to claim 1, further comprising ~~the step~~ of transducing a foreign gene into a cytotoxic lymphocyte.

32. (Original) The method according to claim 31, wherein the foreign gene is transduced using retrovirus, adenovirus, adeno-associated virus or simian virus.

33. (Currently Amended) The method according to claim 1, wherein an expansion [[fold]] ratio of the cytotoxic lymphocyte is high as compared to that of the method for preparing a cytotoxic lymphocyte in the absence of a fibronectin, a fragment thereof or a mixture thereof.

34. (New) The method according to claim 1, wherein expanding a cytotoxic lymphocyte is performed in the presence of both of said fibronectin fragment or mixture thereof and an anti-CD3 antibody.

35. (New) The method according to claim 1, wherein expanding a cytotoxic lymphocyte is performed by incubating peripheral blood mononuclear cells or umbilical cord blood mononuclear cells.

36. (New) The method according to claim 1, wherein said expanding is performed for between 2 to 15 days.